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NUTRITION REVIEWS

February 1949

Nutrition and Arthritis 33
The Fate of Thiamine in the Human Being 35
Pantothenic Acid and Longevity 37
Microdetermination of Riboflavin and its Natural Derivatives 38
Fluorides and Hardness of Tooth Enamel 41
Proteins of Different Biologic Values and Amino Acid Excretion 44
Tooth Decay and Diet in Hawaii 46
Biochemical Aspects of Pteroylglutamates 49
Utilization of Protein Hydrolysates 50
Ascorbic Acid Metabolism in Vitamin A Deficient Rats 52
Nutrition in Adolescence 55
Vitamin Therapy of Gastritis in Man 56
Role of Pyridoxine in Transamination 57
Oxidation of Glucose in Normal and Alloxan-Diabetic Rats 58
Ocular Changes in Deficiencies of Riboflavin or Tryptophan 60
Activity of Pteroylheptaglutamate in Sulfa-Treated Rats 61
Notes 63
William John Dann 63
Vitamin Fortification of Milk 63
Folic Acid and Neurologic Changes in Pernicious Anemia 63
Recent Nutrition Books 64

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NUTRITION REVIEWS

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NUTRITION AND ARTHRITIS

Few conditions have the wealth of medical folklore and number of unconfirmed theories that are encountered in the field of rheumatic diseases. High on the list is the belief that in some way diet has something to do with the causation and cure of many forms of arthritis. Some of these ideas antedate the clearcut separation of the various forms of joint disease into clinical entities, such as the interdiction of meat or "acid" foods due to a confusion of other forms of arthritis with gout. But the failure to find a specific etiology, and the necessary empiricism of treatment for the most common forms of chronic arthritis have made it a fertile field for speculation, and some investigation, regarding metabolic and nutritional factors. As in any chronic disease subject to spontaneous exacerbations and remissions, changes in clinical course associated with changes in diet have led to erroneous conclusions due to the failure of the observers, lay and professional, to distinguish between coincidence and cause and effect.

Rheumatoid arthritis, the most serious of the common chronic joint diseases, has been the object of the most intensive search for a nutritional or metabolic aberration. Patients with this disease usually lose weight and often present an obvious nutritional problem. Yet careful studies of carbohydrate, fat, and protein metabolism have shown no abnormalities which can be considered of etiologic significance. The minor delays in carbohydrate utilization which many of these patients show in the glucose tolerance test can be eliminated in the great majority of cases by feeding a high carbohydrate diet for a few days prior to testing. These patients do not have excessive protein wastage, and positive nitrogen balance can be obtained with an adequate intake of both calories and protein. The hypothesis that a poorly defined sulfur deficiency was important in this disease was thoroughly exploded by R. H. Freyberg, W. D. Block, and M. F. Fromer (*J. Clin. Invest.* **19,** *423 (1940)*) who found no evidence of sulfur deficiency or abnormality in sulfur metabolism, and no biochemical or metabolic need for, or benefit from, sulfur medication in the treatment of arthritis. M. W. Ropes, E. C. Rossmeisl, and W. Bauer (*Ibid.* **22,** *785 (1943)*) found no major alteration of calcium and phosphorus metabolism, although there did appear to be an increased rate of turnover of these minerals, and a small increase in calcium excretion.

A decade ago it appeared that advances in knowledge of the vitamins might solve some of the problems of rheumatoid arthritis. Since the primary pathology of the disease is in the connective tissue, ascorbic acid was particularly implicated because of its importance in intercellular tissue. Low concentrations of ascorbic acid were found in the plasma of many patients with this disease, but the clinical course of the arthritis was unaffected by correction of the biochemical deficiency (R. H. Freyberg, *J. Am. Med. Assn.* **119,** *1165 (1942)*). Since then, the gap between such biochemical evidence of lack of saturation of body stores with a particular vitamin and a clinically significant impairment of body function due to the deficiency has been more widely appreciated. In a somewhat similar fashion, the frequent subclinical and less common frank deficiencies of the vitamin B-complex seen in this disease have been accepted as accompaniments or consequences of a chronic constitutional disease, rather than a cause (T. B. Bayles, H. Richardson, and F. C. Hall, *New Engl. J. Med.* **229,** *319 (1943)*).

The use of massive doses of vitamin D

and related steroids in arthritis, ranging from 100,000 to 500,000 I.U. daily, rests entirely on an empirical basis and can in no way be considered as correcting a deficiency. Those who have attempted to evaluate its use under controlled conditions emphasize its limited value, if any (Freyberg, *J. Am. Med. Assn., loc. cit.*); R. H. Boots, *Ibid.* **123,** *857 (1943)*; C. H. Slocumb, *Ann. Int. Med.* **16,** *241 (1942)*). The exploitation of this form of treatment has given clinicians and pathologists their best opportunity to date of observing the effects of acute and chronic vitamin D intoxication in man (G. W. Covey and H. H. Whitlock, *Ibid.* **25,** *508 (1946)*; J. M. Bauer and R. H. Freyberg, *J. Am. Med. Assn.* **130,** *1208 (1946)*).

More general approaches to the problem of diet and rheumatoid arthritis have also given essentially negative results. Bayles, Richardson, and Hall (*loc. cit.*) using the dietary history method, found the diets of 31 patients with rheumatoid arthritis during the year prior to onset of symptoms apparently the same as that of a cross section of families in the same area. In more than half the subjects, intake of minerals and certain vitamins was below the level recommended by the National Research Council. They concluded that a deficiency in the average diet could not contribute to the onset of rheumatoid arthritis, although the possibility of an increased total requirement in these patients could not be ruled out. W. Bauer (*J. Am. Med. Assn.* **104,** *1 (1935)*) evaluated the following diets in rheumatoid arthritis: (a) low carbohydrate, (b) low caloric, (c) alteration of acid-base balance, (d) omission of "acid" fruits and vegetables, (e) restriction of servings at each meal to one type of foodstuff, (f) eliminating food to which the patient is allergic, and (g) a low protein diet. He concluded that there is no good evidence for the use of any of these in the patient with rheumatoid arthritis. The "raw food diet" probably owes whatever effect it may have to its low sodium and high potassium content (A. A. Holbrook, *Ann. Int. Med.* **20,** *512 (1944)*). S. D. Jacobson, B. Leichtentritt, and R. H. Lyons (*Am. J. Med. Sci.* **204,** *540 (1942)*) have demonstrated temporary improvement in joint swelling and mobility associated with shifts in body water induced by altering the degree of retention of the sodium ion.

Despite these failures to demonstrate any etiologic role of nutritional deficiencies or metabolic errors in this disease, attention to the nutritional state of the patient is often an important part of the management of rheumatoid arthritis. Sound dietary practices, common to the management of any chronic systemic disease, are necessary to restore caloric balance, provide favorable conditions for rebuilding of muscle, and correct any specific deficiencies which may be present. A diet liberal in calories and protein and high in vitamin and mineral content is usually indicated. Adequate justification for protective use of polyvalent vitamin preparations at supplementary levels of dosage is provided by the infrequent occurrence of clinical deficiencies and the studies suggesting a moderate increase in requirements for certain vitamins accompanying this disease. There is no evidence that the patient with rheumatoid arthritis needs a diet differing in any significant respect from the optimum or ideal diet. Yet it is often of considerable importance to see to it that he actually gets it, and eats it.

Osteoarthritis, the degenerative joint disease of older persons, has been included in some of the studies cited above with essentially the same negative conclusions. As this disease commonly affects the weight-bearing joints and is usually seen in obese individuals, reduction of weight by dietary measures is frequently indicated.

Gout, an uncommon but by no means rare disease, is the only form of arthritis in which a definite error of metabolism has been demonstrated. Modern clinical experience does not support the classic concept that this is an affliction of those addicted to overindulgence in food and drink. Despite the

association of this disease with hyperuricemia, "a careful survey of the past and present literature reveals the astonishing fact that conclusive evidence pertaining to the effect of dietary regulation on clinical gout is entirely lacking" (W. Bauer and F. Klemperer, in G. G. Duncan's "Diseases of Metabolism, *Ed. 2, Chapt. XII, p. 638, W. B. Saunders Co., Phila., Pa. (1947)*). There is accumulating conclusive evidence that the metabolic error involved is hereditary in nature (C. J. Smyth, R. M. Stecher, and W. Q. Wolfson (*Science* **108,** *514 (1948)*). Therefore, diet cannot be a factor in the primary etiology of gout. Moreover, the influence of dietary regulation on the course of the disease is open to question. Drastic curtailment of purine intake can be obtained only by a monotonous diet of a few vegetables and cereals, eggs, and dairy products. Caloric restriction is often indicated, and the few foods with unusually high purine content are readily eliminated.

In summary, there is no diet or essential food factor which is known to be involved in the causation of most forms of arthritis, and no diet or vitamin which so far as known can be expected to cure it. As is true of many other measures utilized in the management of disease, the diet must be adapted to the general condition of the individual patient and to the type of joint disease which he presents. A good wholesome nutritious diet is an important part of present day preventive and curative therapy.

WILLIAM D. ROBINSON
Rackham Arthritis Research Unit
University of Michigan
Ann Arbor, Michigan

THE FATE OF THIAMINE IN THE HUMAN BEING

Several investigators have shown that the excretion of thiamine at adequate levels of intake by the human being is approximately of the same order whether the vitamin is administered orally, intramuscularly, or intravenously. However, at a higher level of intake, absorption, as indicated by urinary excretion, is not as complete and differs somewhat depending on the dose and method of administration. According to A. S. Schultz, R. F. Light, and C. N. Frey (*Proc. Soc. Exp. Biol. Med.* **38,** *404 (1938)*) the maximal dose of thiamine which can be completely absorbed is 2 to 3 mg. Others state that the limit of absorption of a single oral dose appears to be about 5 mg. However, Schultz, Light, and Frey have found an increased quantity of thiamine in the stools after administration orally of a dose of 5 mg.; when an additional quantity of 5 mg. is given orally almost all of the additional vitamin is recovered in the stools. In the human being relatively little absorption of thiamine has been noted when the vitamin is given in retention enemas. The most rapid excretion in the human being is noted within three to eight hours after administration of a single dose of 5 mg. with food (D. Melnick, H. Field, and W. D. Robinson, *J. Nutrition* **18,** *593 (1939)*; M. Jowett, *Biochem. J.* **34,** *1348 (1940)*). From these reports it would seem that the absorption of thiamine in the human being is confined largely to the upper portion of the intestinal tract, whereas in aged persons the absorption apparently is extremely limited throughout the entire intestinal tract (H. A. Rafsky and B. Newman, *Gastroenterology* **1,** *737 (1943)*).

T. E. Friedemann, T. C. Kmieciak, P. K. Keegan, and B. B. Sheft (*Gastroenterology* **11,** *100 (1948)*) have attempted to define the limits of absorption and destruction of thiamine in the body of normal human subjects when the substance is given orally in doses within the therapeutic range.

The subjects employed were medical students and laboratory workers, varying in

age from 20 to 40 years. Urine was collected in gallon-sized amber glass bottles which contained 5 ml. of twentieth-normal sulfuric acid. This yielded an approximate tenth-normal acidity in the twenty-four hour sample. At this level of acidity, bacterial growth was inhibited and loss of thiamine was not noted within two weeks when the samples were refrigerated. Each daily sample was either analyzed immediately or a one-tenth aliquot was transferred to a bottle that contained a composite sample. The latter was kept in a deep-freeze unit until ready for analysis. All stools were collected in tarred containers which likewise were kept in a deep-freeze unit. In some experiments, individual samples were collected. The total amount of thiamine was determined by the modified method of B. C. P. Jansen (*Rec. trav. chim.* **55,** *1046 (1936)*). Free thiamine was determined similarly except that the digestion with takadiastase was omitted.

The studies included many experiments, and detailed data are well tabulated in the article.

Data from the experiments done on 14 human subjects demonstrate the limited absorption of thiamine from the intestinal tract. Supplements of 1.0 mg. taken with meals three times each day are apparently completely absorbed, since the thiamine content of the feces is not increased. If one considers the vitamin content of the diet, perhaps the maximum of complete absorption and, therefore, the maximal economic level of the vitamin, is about 5 mg. per day. When the daily intake is increased, the rate of absorption rises somewhat and relatively larger quantities are excreted in the feces. The maximal absorption of 8 to 14 mg. is obtained with daily supplements of 20 to 40 mg. given in three or four divided doses with food. When thiamine was given one hour before meals the excretion was not increased by the simultaneous administration of bile salts, hydrochloric acid, or hydrochloric acid plus bile salts.

These reports are of interest clinically because they indicate that oral doses of more than 5 mg. of thiamine are largely wasted. Thus, it would seem that when large doses are necessary or a high concentration of thiamine is desired in the blood, the vitamin should be administered parenterally.

After ingestion of a single dose of a solution that contained 50 mg. of thiamine, a subject excreted 4.58 to 3.81 mg. of thiamine in the urine on the next two days. Less thiamine was excreted (0.93 and 1.28 mg.) on the third and fourth days, and, thereafter, it was excreted in rapidly diminishing quantities. However, the excretion of thiamine continued above basal levels up to the twelfth day. Of interest was the finding that excretion of thiamine in the urine diminished rapidly before any of the vitamin was recovered in the stools.

Careful studies of the feces led the authors to state that about 4 mg. of thiamine are destroyed in the intestinal tract and tissues each day in subjects who have at least one normal bowel movement a day. Apparently the destruction occurs largely in the tissues because approximately equal quantities are not recovered after a single oral dose of 50 mg. Secondly, the destruction is about the same when daily supplements of up to 40 mg. are given in three or four divided doses over an extended period. In the latter instance, the average daily excretion of thiamine in the feces may vary from 0.3 to 1.8 mg. with 7 mg. of supplement, to 28 to 30 mg. with 40 mg. of supplement. These results suggest an approximately constant rate of destruction of thiamine in the tissues when quantities of about 6 to 14 mg. per day are absorbed. This constant maximal destruction, which is attained at levels of intake well above saturation, is due perhaps to the rapid rate of renal excretion, which tends to maintain a relatively low and constant level of thiamine in the plasma. There is little doubt that some of the vitamin is destroyed in the intestinal tract.

These results were compared with data

from other experiments and would seem to suggest that the colon is the site of greatest intestinal destruction.

Thiamine mononitrate was absorbed, excreted, and destroyed at rates similar to that of thiamine hydrochloride.

Thiamine would appear to belong to those groups of substances which are absorbed relatively poorly in the gastrointestinal tract of man. The nature of the barrier-mechanism which prevents further absorption is not understood.

PANTOTHENIC ACID AND LONGEVITY

Interesting observations associating pantothenic acid with longevity have been reported using bees and fruit flies as experimental organisms. Apparently, bee larvae fed on royal jelly during their complete life cycle develop into queen bees. The bee larvae which obtain this special potion only for the first few days of life, develop into the worker bees. The queen bee also receives royal jelly during the heavy egg-laying periods. Queen bees are known to live about five years, while the worker bees usually live only one summer. Naturally, royal jelly has been considered as a possible factor in the longevity of the queen bee.

Royal jelly is a viscous fluid with a flower-like odor that is secreted by the pharyngeal glands of young worker bees. According to R. M. Melampy and D. B. Jones (*Proc. Soc. Exp. Biol. Med.* **41,** *382* (*1939*)) it is composed of about 66.0 per cent water, 12.3 per cent protein, 5.5 per cent total lipoids, and 12.5 per cent total reducing substances; ash, 0.82 per cent and undetermined substances 2.84 per cent. Others (G. F. Townsend and C. C. Lucas, *Biochem. J.* **34,** *1155* (*1940*)) have published reports concerning the chemical separation of the various components of royal jelly. Vitamins E, A, and C are practically absent from royal jelly but it is a fair source of thiamine. It is apparently the richest known natural source of pantothenic acid (G. Kitzes, H. A. Schuette, and C. A. Elvehjem, *J. Nutrition* **26,** *241* (*1943*); P. B. Pearson and C. J. Burgin, *Proc. Soc. Exp. Biol. Med.* **48,** *415* (*1941*)). Kitzes, Schuette, and Elvehjem, using microbiologic assays on one sample of royal jelly obtained for pantothenic acid 320, riboflavin 28, niacin 111, pyridoxine 10.2, biotin 4.1, and folic acid 0.5 microgram per gram on a dry basis. Royal jelly also is said to have marked bacteriostatic and bactericidal properties (C. S. McCleskey and R. M. Melampy, *J. Bact.* **36,** *324* (*1938*)).

In two articles, T. S. Gardner (*J. Gerontology* **3,** *1, 9* (*1948*)) has postulated that if certain chemical components in royal jelly caused the queen bee to live longer than the workers, it might be possible, by the use of royal jelly, to extend the span of life of a related form of life, such as the fruit fly. The design of his experiment was such as to test various fractions of royal jelly separately and also to strain out any compound or compounds that might give promise of being factors of longevity. The fruit flies used were Drosophila melanogaster oregon. Cultures were prepared in the usual manner and were kept practically free of bacteria because it is known that pantothenic acid is a good promoter of the growth of bacteria. The fruit fly cultures were laid on a yeast-banana medium. The newly emerged imagoes were transferred to test bottles and observed daily. When the imagoes were ready to emerge from pupae in the test bottles the flies were transferred to fresh bottles containing the same composition. Dates of death were recorded and an arithmetic average was computed. Fifty to 100 flies were used to ascertain the average length of life in each instance. Ten to 15 flies were allotted to each bottle and the temperature in the bottles was maintained

at 30°C. The average length of life was 13 to 14 days. The test fractions of royal jelly were added in solution to the hot medium and then further sterilized at 100°C. for three minutes. The only exception was the test fraction which contained pantothenic acid. The calcium pantothenate was added in sterile cold water to cold mediums which had been sterilized previously. This was considered advisable because of the instability of free pantothenic acid when subjected to heat and, especially, to hot acid solutions.

The royal jelly was dried and separated according to the chemical procedures of Townsend and Lucas (*loc. cit.*). Small quantities of dehydrated royal jelly had no effect on the span of life but large quantities had beneficial effects. The over-all ether insoluble fraction had no effect on the span of life but the ether soluble fraction decreased the average span. The fraction that consisted of carbohydrates and proteins had no appreciable effect on the span of life in the quantities tested. The fraction that contained phenols, waxes, and phospholipids slightly decreased the average length of life while the fraction with sterols and glycerides had no effect. The span of life was not affected by small quantities of the fraction that consisted of combined water soluble and insoluble organic acids but large quantities resulted in beneficial effects. The increase in the length of life when pantothenic acid was added was much greater than any experimental error and was considered by the author as a real contribution to the longevity of the fruit fly. Therefore, it was assumed to be one of the factors in royal jelly which aids in prolonging the life of the queen bee.

Also, it has been demonstrated that yeast nucleic acid increases the span of life of white mice when it is fed to them from birth at a level of 25 mg. each day for six days a week (T. B. Robertson, *Australian J. Exp. Biol. Med. Sci.* **5,** *47 (1928)*). Since the effect of yeast nucleic acid, or its soluble sodium salt, on fruit flies had not been investigated, the author considered it advantageous to extend the investigation on fruit flies, which had been carried out using royal jelly, to include biotin, pyridoxine, and sodium yeast nucleate. Again, the same type of fruit fly was employed and the water soluble vitamins and sodium yeast nucleate were added in solution in cold sterile water to cold, previously sterilized culture mediums.

Biotin had no effect on fruit flies in so far as length of life was concerned, but pyridoxine gave an average increase of life of 10.5 per cent and sodium yeast nucleate of 11.3 per cent. However, when powdered pyridoxine and sodium yeast nucleate were used to supplement one another, the span of life was increased by 20.3 per cent; when pantothenic acid was used alone the increase was 27.8 per cent. When pantothenic acid was added to powdered pyridoxine and sodium yeast nucleate the span of life was increased to 46.6 per cent. It is suggested that the favorable effect of the various components in increasing the life span of the fruit fly is due to a synergistic action exerted on each other. This, of course, is greater than that of any test nutrient taken alone.

The evidence that pantothenic acid is a limiting factor for longevity is not conclusive, but it is interesting and suggestive. These investigations also indicate that fruit flies apparently under careful, bacteria-free, and controlled conditions may be used to determine which compounds, if any, might be associated with the aging process.

MICRODETERMINATION OF RIBOFLAVIN AND ITS NATURAL DERIVATIVES

The lack of sufficiently accurate, simplified technics for the determination of riboflavin and its derivatives in small volumes of blood has made it almost impossible to con-

duct satisfactory surveys to study and evaluate the relations of blood levels of riboflavin to the riboflavin intake and to the nutriture of the individual or group with respect to this vitamin. Results with microbiologic methods fail to differentiate between riboflavin and its derivatives and have been somewhat difficult to standardize because of the constant presence of other growth-promoting and growth-inhibiting substances in blood and blood extracts.

O. H. Lowry (*J. Biol. Chem.* **173,** *677 (1948)*) has developed a very sensitive fluorometer which makes possible methods which have the advantages of being simple, rapid, and reproducible to 3 to 5 per cent, and by which riboflavin and its natural derivatives can be differentiated. The fluorescence of as little as 0.1 millimicrogram of riboflavin in 0.5 ml. of solution could be measured with an accuracy of 5 per cent. This was a one thousand fold increase in sensitivity and stability over ordinary types of fluorometers. A "multiplier" phototube (RCA #1P21) was substituted for the phototube in the commercial photofluorometer. A suitable electrical circuit for the operation of this tube was found to be relatively simple to construct. Provision was made for a coarse and fine adjustment which provided a hundredfold range in sensitivity. Fluorescence of the components of the ordinary optical system was found to limit the useful sensitivity to a great extent. Pyrex tubes were substituted for ones of soft glass which gives off a weak fluorescence, especially when the shorter exciting wavelengths are used. Some slight fluorescence was eliminated by the replacement of the original lenses with equivalent Pyrex or quartz ones. Fluorescence of the light filters was a considerable problem which was reduced but not completely removed. This micro fluorometer was tested under various circumstances for its suitability for the determination of the fluorescence of riboflavin and thiochrome and found to be suitable within the recommended ranges for the respective assays.

The use of this micro photofluorometer by H. B. Burch, O. A. Bessey, and O. H. Lowry (*J. Biol. Chem.* **175,** *457 (1948)*) has resulted in methods "for determining (1) both free and total riboflavin in 50 c.mm. of blood serum (25 c.mm. for total alone); (2) the free riboflavin, flavin- mono- and dinucleotide as separate fractions in 0.2 ml. of serum; (3) the total riboflavin in 20 c.mm. of red cells or whole blood; and (4) the total riboflavin in white cells and platelets from 0.1 ml. of blood." The differentiation of the flavin compounds was based on these three properties: (a) in neutral solutions on a molar basis, riboflavin and flavin-mononucleotide exhibit the same fluorescence, while flavin-adenine-dinucleotide was only about 14 per cent as active under the conditions of the analysis; (b) riboflavin is much more readily extracted by benzyl alcohol from aqueous solutions than is flavin-mononucleotide or flavin-adenine-dinucleotide; and (c) flavin adenine-dinucleotide is completely hydrolyzed to flavin-mononucleotide in 5 per cent trichloracetic acid in twenty hours at 37°C. To test the reliability of the described methods, riboflavin and flavin-adenine-dinucleotide were added to human whole blood, serum, and red and white cell suspensions prior to precipitation of the proteins with trichloracetic acid. Filtrates were then analyzed at least in triplicate by the proposed methods. In all cases, quantitative recovery was observed. Similar analyses with rat blood, serum, and cells were equally satisfactory.

The riboflavin content of the serum of 13 supposedly well nourished individuals was determined. The riboflavin content of the white blood cells plus platelets and of the red blood cells was calculated for 12 healthy adults. Flavin-mononucleotide was found to be present in negligible amounts. The average free riboflavin plus flavin-mononucleotide concentration in the serum was found to be 0.8 microgram per cent with a range of 0.3 to 1.3 micrograms per cent. The average total riboflavin content in

serum was determined to be 3.2 micrograms per cent with a range of from 2.6 to 3.7 per cent. The average total riboflavin content of white blood cells plus platelets was 252 micrograms per cent with a range of 227 to 293. A comparison of the analyses of the upper buffy layers containing predominantly platelets with analyses of the lower layers containing mainly white blood cells indicated that there was no appreciable difference between them with respect to their total riboflavin content. The average total riboflavin concentration of red blood cells was 22.4 micrograms per cent with a range of 18.0 to 26.2.

These investigators found that whole blood samples could be stored in the dark at 4°C. for forty-eight hours without any measurable change in the concentration of the individual riboflavin compounds. Serum and red and white cells were stored for several months at 40°C. without change in the total riboflavin content.

An interesting difference was found between the hydrolysis of flavin-adenine-dinucleotide which was added to the serum and that which was originally present. When flavin-adenine-dinucleotide was added to serum, about 80 per cent hydrolyzed in one hour at room temperature; in contrast, no evidence of hydrolysis of the flavin-adenine-dinucleotide which was naturally present in the serum was detectable for many hours. These data would seem to indicate that the flavin-adenine-dinucleotide in serum was combined with protein and that an enzyme system existed capable of splitting uncombined flavin-adenine-dinucleotide but incapable of hydrolyzing the bound form.

To determine how the dietary riboflavin intake was related to blood and urine levels in rats, the blood of rats which had been receiving rations containing 0.5, 1.0, 1.5, 2.0, 3.0, or 10.0 micrograms of riboflavin per gram of diet was analyzed. Additional amounts of riboflavin up to 3.0 micrograms per gram of diet resulted in increased rates of growth. The free riboflavin level of the serum of the rats on the lowest level of riboflavin was negligible (0.14 ± 0.11 micrograms per cent) and increased with each successive increase in ingested riboflavin 3.79 micrograms per cent in the serum of rats fed the highest level. In all groups, the serum flavin-mononucleotide was very low and was not demonstrably affected by the dietary alterations. The concentration of flavin-adenine-dinucleotide in the serum increased much less than the free riboflavin; the maximum increase from the lowest to the highest riboflavin intake was about 40 per cent. The increase in total riboflavin concentration was from 1.55 ± 0.09 to 5.82 ± 0.23 micrograms per cent and reflected primarily the changes in the free riboflavin levels. The total riboflavin content of the white and red cells increased 50 and 60 per cent, respectively, as the amount of riboflavin in the diet was increased. The data on riboflavin excretion in the urine were tabulated on the basis of the number of micrograms of riboflavin excreted per milligram of creatinine. The amount of riboflavin excreted was very low for the rats fed the diets containing between 0.5 and 2.0 micrograms of riboflavin per gram and varied between 0.14 and 0.20 micrograms per milligram of creatinine. When the level of riboflavin in the diet was increased to 3.0 micrograms per gram, the urinary excretion increased to 0.75 micrograms per milligram of creatinine. The further increase of ingested riboflavin to 10.0 micrograms per gram, resulted in an increase in the urinary excretion figure to 13.8.

Three human subjects restricted their riboflavin intake to about 0.4 mg. per day for four days. The total riboflavin concentration in the serum decreased from the initial levels by 8, 16, and 25 per cent, while the urinary excretion during a five hour collection period was about one-fourth that of a similar period before the short depletion phase. On the morning of the fifth day,

each subject was given 2 mg. of riboflavin with 600 ml. of water and a few crackers. The greatest increase in serum riboflavin was observed thirty minutes after ingestion. An abrupt decrease was noted when the second blood sample was taken; by the end of the second hour the riboflavin values of the serum approached the initial values. The increase in the urinary excretion was in the neighborhood of thirty to forty-five times the initial level during the first hour after ingestion and remained high for a more prolonged period than the serum levels.

These methods and the results which have been obtained through use with rats and human beings appear to be encouraging. Admittedly the experiments to study the effect of varying intakes have been preliminary ones; the results, especially with the determination of free riboflavin, seem to indicate that there are definite relations between riboflavin intake and blood levels which may be of the greatest value as diagnostic criteria for evaluating the state of nutrition of individuals or groups with respect to riboflavin.

FLUORIDES AND HARDNESS OF TOOTH ENAMEL

The relation of fluorides in the drinking water supply and of topically applied fluorides to the incidence of tooth decay has been the subject of much interest in the public health, nutrition, and dental fields and has been the subject of numerous previous reviews (*Nutrition Reviews* **1,** *110, 182 (1943);* **2,** *35, 97, 149 (1944);* **3,** *115 (1945);* **4,** *73, 80, 145 (1946);* **5,** *68, 199, 322, 348 (1947)*). The mechanism of action of fluorides as possible caries-preventing agents to be widely used as public health measures is, at present, the subject of much controversy with little supporting experimental evidence for any of the numerous hypotheses. The major chemical component of enamel is thought to be hydroxyapatite and some investigators believe that the soluble fluorides when in intimate contact with the enamel will react in such a way that the hydroxy groups are replaced by fluoride ions to form the less soluble fluorapatite. It is unknown, however, whether the change which occurs is accomplished by an absorption process or by a change in the space lattice or by a combination of both processes.

R. W. Phillips and M. L. Swartz (*J. Am. Dental Assn.* **37,** *1 (1948)*) have made extensive tests on about eighteen hundred extracted human teeth to determine whether the application of fluorides to enamel surfaces might be accompanied by definite changes which would be reflected in the hardness of these areas. Such physical changes might accompany the reduced solubility of enamel in acid which has been demonstrated to be induced by the topical application of fluorides (J. F. Volker, *Proc. Soc. Exp. Biol. Med.* **42,** *725 (1939)*). A precise method for testing the hardness of enamel before and after the use of various test solutions involved the use of a precision electronic instrument, the Tukon tester. Basically this instrument was used to measure hardness of materials by applying under carefully standardized conditions a Knoop indenter, a small diamond-shaped point, against a selected area of the surface being tested. Measurement of the dimensions of the indentation made in the surface by the Knoop indenter coupled with the constants of the indenter and the applied load can be expressed as the Knoop hardness number of that surface.

To have a hardness test which was highly standardized to reduce experimental error, these investigators recommended that the greatest precaution had to be taken in the selection of teeth, in the preparation of the smooth flat surface to be indented, and in all phases of the measurement of the indentation. Before any treatment of the tooth

with fluorine, two indentations were made on the prepared surface under identical conditions; the hardness number of the untreated tooth was computed as the average of these two determinations. Following treatment with the test solution, three additional indentations were made, one between the two original indentations and one on either side; the after-treatment hardness number was the average of these three values. This procedure of indentation was considered to overcome the possibility of any effect of the hardness variation within any tooth upon the accuracy of the results. Since original hardness numbers vary greatly from tooth to tooth, hardness changes for any tooth or group of teeth were computed and expressed in terms of percentage change from the original rather than as the direct loss or gain in actual Knoop numbers.

The temperature of all test solutions was maintained at 99 ± 0.5°F. with constant agitation during the period in which teeth were being treated. After removal from the test solution, the tooth surface was cleaned carefully with a camel's hair brush dipped in soap and water. The surface was then rinsed thoroughly with water and dried. Thirty teeth were prepared and their hardness measured. Each tooth was then immersed for five minutes in 10 ml. of a 1:500 solution of sodium fluoride buffered to a pH of 4.0 with glacial acetic acid. After washing and drying, the hardness of these teeth was redetermined and found to have increased 5.1 per cent. Thirty teeth were similarly treated with a 1:500 solution of stannous fluoride buffered to pH 4.0. The average hardness change in this case was 7.1 per cent. In the opinion of the investigators, these increases in hardness indicated that there had been a definite physical change in the enamel surface which they believed reflected a chemical change in the apatite molecule. On the basis of present knowledge, these increases in hardness must not be interpreted to mean that the caries susceptibility of the tooth has been altered, but are important as indications that the surface has been affected sufficiently by immersions in solutions of sodium fluoride and stannous fluoride to produce physical and possibly chemical changes. A film on the selected surface was noted after immersion in the fluoride solutions which adhered tenaciously enough to resist the washing and rinsing procedure. To test whether this deposit affected the hardness value, an experiment was made with fifteen teeth which were immersed for twenty minutes in a 1:500 stannous fluoride solution. After immersion, one half of each prepared test surface whose hardness value had been determined previous to fluoride treatment was cleaned by intensive brushing to remove all the deposit. Hardness tests were made on the area with the deposit and that from which the deposit had been removed. The increase in hardness for the two areas was identical within the range of experimental error indicating that the hardness increase was not attributable to the deposit.

Experiments to test the effect of exposure time indicated that maximum increase in hardness after immersion in stannous fluoride solution was not attained until the end of twenty-five to thirty minutes' immersion. In another study, the effect of saliva on the ability of fluoride solutions to increase hardness was made by immersing the teeth in a solution of "synthetic" saliva for twenty minutes at 99°F. prior to the sodium fluoride immersion. Under these circumstances, the increase in hardness was somewhat lower than when the teeth were dry at the time of immersion into the fluoride solution. This is not surprising, since the physical nature of saliva would be expected to reduce the contact between the fluoride solutions and the enamel surface. The depth of penetration of the change in hardness was believed to be appreciable since three minutes of abrasive brushing with pumice after protection by immersion for five minutes in fluoride solution were necessary before unprotected enamel was exposed. After twenty-five minutes of immersion, four

minutes of brushing under the same conditions were insufficient to reach an area of lower hardness than the protected surface. Longer brushing periods could not be used as more prolonged abrasion rounded the test area too much to permit an accurate hardness test.

The next step was to test whether the immersion of teeth in solutions of fluorides and other solutions would alter the ability of acids to soften the enamel. Thirty-two teeth were immersed in 10 ml. of 0.2 molar acetic acid, buffered to pH 4.0 with sodium hydroxide for five minutes. The average reduction in hardness for these teeth was 17.6 per cent. These teeth were resurfaced and new hardness measurements made on the same test area. The teeth were then immersed for five minutes in the 1:500 stannous fluoride solution. The average increase in hardness was 7.1 per cent. After fluoride immersion, these teeth were immersed in acetic acid of the same concentration as above. The decrease in hardness was 5.5 per cent, i.e., the hardness after etching with acetic acid still averaged 1.6 per cent greater than before stannous fluoride immersion. The total difference between the hardness measurements made after acetic acid immersion without fluoride treatment to the hardness values after acetic acid immersion of the same teeth after fluoride treatment was from −17.6 to +1.6 per cent or a difference of 19.2 per cent. Similar tests made with solutions of lead fluoride, uranyl nitrate, and sodium fluoride in between acetic acid immersions indicated lower differences but still protection in each case of 15.4, 12.1, and 7.9 per cent, respectively. Solutions of zinc fluoride, copper fluoride, lead nitrate, silver nitrate, and sodium acetate did not alter the amount of softening by acetic acid.

It is difficult to relate the findings in this investigation to what might occur in human teeth which are systemically exposed to fluorides throughout their development and maturation. Because of the great variation in hardness values for human teeth, the hardness tests at present appear to be insufficiently standardized to test whether teeth which have been exposed to fluorine throughout development and maturation are, on the average, harder than those which were not exposed. Possibly if fluorine determinations could be made routinely on the teeth used for hardness tests some correlation between fluorine content and hardness might be possible. Two of the most valuable uses of a hardness-testing apparatus in dental research, would be the elaboration of a standardized method for testing hardness of very small teeth such as those of the rodents and for testing the hardness of the teeth *in situ* in the oral cavity of man and experimental animals over long periods of time from immediately after eruption to old age under both normal and abnormal conditions of nutrition and endocrine balance. The requirement for an absolutely flat surface for the hardness test and the accuracy with which the tooth must be positioned for the test and the accuracy with which measurements must be made makes it almost impossible to devise such a test with the apparatus now available.

This investigation appears to show with considerable accuracy that physical changes can and do occur when enamel surfaces are exposed to certain test solutions such as stannous fluoride, lead fluoride, uranyl nitrate, and sodium fluoride. Possibly the exposure of teeth to certain of these compounds during development results in more striking changes of the same type. Of considerable interest is the fact that materials other than compounds containing the fluoride ion have the same effect on hardness and that the fluorides themselves have different effects at the same concentration that are apparently not affected by the solubility of the compounds. These findings need to be tested in considerable detail with experimental animals to determine whether the effect of caries susceptibility is in any way related to the difference in hardness values.

PROTEINS OF DIFFERENT BIOLOGIC VALUES AND AMINO ACID EXCRETION

H. E. Sauberlich and C. A. Baumann (*Nutrition Reviews* **5,** *213 (1947)*) have described methods of microbiologic assays for sixteen amino acids which are satisfactory for use in studying the urinary excretion of these amino acids by the mouse and rat. A series of experiments has been conducted by E. L. Pearce, H. E. Sauberlich, and C. A. Baumann (*J. Biol. Chem.* **168,** *271 (1947)*) to determine whether the urinary excretion of amino acids by mice varied with the biologic value of the protein fed.

In the first experiment, weanling mice weighing 10 to 12 g. were fed diets of the following general composition: protein 10 per cent, corn oil 5 per cent, Wesson's salt mixture 4 per cent, and cerelose 81 per cent. Adequate supplements of the required vitamins were incorporated into this basal mixture. The proteins used in this experiment were casein or fibrin or oxidized casein; the latter was fed alone or supplemented with methionine, tryptophan, and cystine. When the protein in the diet was casein or fibrin, the mice gained about 2.5 g. per week. When finely ground oxidized casein was fed with adequate supplements of methionine, tryptophan, and cystine, the mice gained as much as 3.0 g. per week. When either methionine or tryptophan was omitted from the latter diet, the animals lost weight at rates ranging from 0.5 to 2.0 g. per week and some died after two or more weeks. After fifteen or more days on the various diets, the mice were placed in metabolism cages in groups of 3, and the urine collected under toluene for periods of twenty-four to forty-eight hours.

When either fibrin or casein was used as the source of protein in these diets, the mice excreted very low percentages of the amino acids ingested. When ordinary casein was fed for a period of forty-four days before urine collection, the percentages of ingested amino acids which were excreted were found to vary from 3.2 for methionine to 8.9 for histidine. When a ration containing fibrin at a level of 20 per cent was fed for forty-four days before the urine was collected, only 0.8 per cent of the ingested arginine was excreted in the urine while as much as 5.4 per cent of the ingested histidine were excreted. The mean of the different amino acids excreted after complete proteins were ingested was only 2.9 per cent. The levels of amino acids determined in these analyses represented only the amounts of amino acids which were available to the microorganisms and does not include those amino acids which were bound in such peptides as to be unavailable to the microorganisms.

However, the mice which were consuming a diet with an inadequate source of protein, excreted in microbiologically available form abnormally high amounts of all other amino acids than the ones in which the proteins were deficient. On the tryptophan deficient diet which contained oxidized casein plus methionine and cystine as the source of protein, the percentage of ingested amino acids appearing in the urine after fifteen days varied from 12.8 per cent for proline to 48.3 per cent for histidine, with the other amino acids being excreted in intermediate percentages of the ingested amounts. Similarly, when the protein source was deficient in methionine, mice excreted 9.9 per cent of the ingested aspartic acid and 37.5 per cent of the ingested histidine. After more prolonged experimental depletion periods, the percentage excretion ranged from 24.6 for glutamic acid to 86.5 per cent for histidine. The mean excretion was 24.5 per cent in the latter experiment. When oxidized casein was supplemented with methionine, tryptophan, and cystine, the excretion of all amino acids except those added in free state was reduced to 10 per cent or less of the amount ingested. Those added in the free state were present in the urine in somewhat higher amounts probably due to a little spilling of the ration into the urine. The excretion of

microbiologically available amino acids was essentially the same on properly supplemented oxidized casein as when untreated casein was fed at a comparable percentage of the diet. This strongly suggested that the high excretion of amino acids on the deficient diets was due to the deficiency of the missing, essential amino acids and not to any inherent property of the oxidized casein. Further emphasis of this point was given by the excretion data obtained when arachin, which is inadequate but not completely deficient in methionine, was fed. When feeding of the arachin diet was prolonged to sixty-three days, the percentages of ingested amino acids excreted in the urine increased to 8.8 for aspartic acid and to 20.5 per cent for lysine.

Roughly one half of the amino acids excreted in the urine from mice fed complete proteins existed in bound form, i.e., in such chemical combinations as were unavailable to the microorganisms used in the assays. Urine from mice fed incomplete protein contained approximately the same amounts of bound amino acids as the urine from mice fed complete proteins. Since the abnormal urine contained greatly increased amounts of "free" amino acids, the percentages of the total existing in the bound form were consequently relatively lower.

In later experiments, H. E. Sauberlich, E. L. Pearce, and C. A. Baumann (*J. Biol. Chem.* **175,** *29 (1948)*) expanded their investigations to include other proteins and preliminary studies with the rat. In the first group of experiments, mice were used to test the effect of the ingestion of casein, egg albumin, lactalbumin, fibrin, and arachin at a level of 8 or 10 per cent on the urinary excretion of amino acids. In the second series, the effect of oxidized casein supplemented with tryptophan or methionine or both was tested. In addition, the food consumption of one group of rats was restricted to a comparable amount of ration to that voluntarily consumed by the animals fed the ration with oxidized casein. With mice on experiment for forty days, the mean percentage excretion of amino acids as determined by the average of five values made at intervals throughout the experiment was 1.0 for lactalbumin, 1.5 for egg albumin, 2.7 for fibrin, and 3.4 for untreated casein. However, the mean urinary excretion value determined in similar fashion for arachin was 4.7 per cent. When these values were compared with the rates of growth, those proteins which promoted the most rapid rates of growth were metabolized with the least loss of microbiologically available amino acids in the urine.

In the experiments with rats, the determinations of excreted amino acids were limited to glutamic acid, histidine, lysine, phenylalanine, and valine. The average amount of ration consumed by the rats fed the ration with unsupplemented oxidized casein was 4.4 g. per day in contrast to 8.4 g. for the rats fed the ration with oxidized casein supplemented with tryptophan and methionine, and 9.6 g. for the rats fed the ration with untreated casein. Despite their reduced food consumption, the rats fed the ration with deficient proteins excreted at least as much of the measured amino acids as the rats fed the more complete proteins. Apparently the mixture of oxidized casein plus tryptophan and methionine was not as adequate as untreated casein, for the percentages of ingested amino acids excreted by the former group exceeded those of the latter. The low excretion of "free" lysine in the urine suggested that lysine may have been the limiting factor in this regimen.

When the food intake of rats on the ration with untreated casein was restricted, the percentage of "free" amino acids excreted in the urine was much lower than on the same ration provided ad libitum. The actual mean percentage values for amino acids excreted were 0.5 per cent of the ingested amino acids for the restricted group in contrast to 1.6 per cent for the ad libitum controls. This would seem to indicate an ability of the animal body to economize

under stress of this kind. In contrast, the actual mean percentage value of amino acids excreted for the group of rats fed the ration with supplemented, oxidized casein was 5.5 per cent.

Determinations of the amino acids in hydrolyzed urine indicated that the effects of the various proteins upon the excretion of total amino acids were similar to their effects on the excretion of "free" amino acids. The highest percentages of total ingested amino acids excreted were observed on the diets containing the complete proteins. The rat appears to excrete excess acids in both the "bound" and "free" form whereas the mouse excretes them largely in the form which is immediately available microbiologically.

B. S. Schweigert (*Proc. Soc. Exp. Biol. Med.* **66,** *315 (1947)*) has made similar studies of the effect of tryptophan deficiency in the rat on the urinary excretion of amino acids. The diet used was essentially the same as in the previous experiment and contained 12 per cent oxidized casein supplemented with adequate amounts of cystine and methionine. Approximately twice as high amounts of ingested amino acids were excreted by the tryptophan deficient animals as were excreted by those receiving adequate tryptophan. A similarly high excretion of amino acids was observed in a group of riboflavin deficient rats. No increase was observed in niacin deficient rats. After acid hydrolysis of the urine, a two to fourfold increase was observed in the values for histidine, arginine, threonine, and phenylalanine indicating that a high amount of the amino acids excreted was in unavailable form.

These data indicate the relation of the biologic value of proteins and the excretion of amino acids. It is unknown at present whether application of these findings to human beings would yield data of importance for further understanding of protein metabolism and amino acid requirements. If the data to be obtained merited the additional personnel and equipment, the simplicity of the methods for determining the various amino acids would indicate that such determinations could be made routinely and conclusively in a survey of a large human population.

TOOTH DECAY AND DIET IN HAWAII

The importance and etiologic relation of the various factors which have been implicated in the etiology of tooth decay have been the subject of several previous reviews (*Nutrition Reviews* **5,** *68, 178 (1947);* **6,** *43, 196 (1948)*).

N. P. Larsen (*J. Am. Med. Assn.* **137,** *832 (1948)*) has reported the results of a dental survey among 866 children between the ages of 6 months and 18 years living on three sugar plantations in Hawaii. These children were stated to be of vivacious health and were from several racial backgrounds. Most were of Japanese or Filipino parentage; roughly 100 were of mixed blood, the remainder were Chinese, Caucasian, Puerto Rican, Korean, or Hawaiian. Only 4 Hawaiians were included as there are apparently only relatively few racially pure, surviving Hawaiians living in the Islands. The caries experience of a second group of 180 children attending three selected kindergartens in Hawaii was determined. In addition, 104 children, who had recently arrived from the Philippine Islands, were studied. These latter children had suffered malnutrition and to some extent even starvation during the last war. They were still undernourished, anemic, and heavily infested with intestinal parasites. A complete physical examination was done on most of these children as well as a dental examination with mouth mirror and explorer but un-

supported by roentgenograms. A dietary history was taken by trained personnel.

The percentage of carious teeth for the plantation children was 41 and for the kindergarten children 55. In contrast, the 104 children among the recently immigrated Filipinos had only 11 per cent carious teeth. These data were in contrast to the state of nutrition of the groups. One possible exception, of course, was the group of 4 Hawaiians, 25 per cent of which were stated to be caries-free, but despite the sound teeth of the one individual, 70 per cent of all teeth of the group were carious. These percentages are virtually meaningless because of the small size of the group. This contrast between the new Filipinos and the indigenous children is striking; thus 49 per cent of the recently arrived Filipinos had decay-free teeth while only 5.6 per cent of the Filipinos who had been brought up in the Hawaiian Islands illustrated this good state of dental hygiene despite the fact that the children in the latter group were presumably in more robust health than the former. The author then attempted to discover what factors other than actual state of health at the time of examination might be playing a role in the marked difference in incidence of dental caries between these groups of children.

With respect to diet, two groups of children containing representatives of all racial extractions in unknown ratios were studied. The first group was on a plantation in which the usual plantation diet was consumed. The second group, of 250 children, on another plantation a few miles away, had been attending a teaching diet clinic since 1930. There was a somewhat lower incidence of dental caries in the group living on the second plantation; the only striking difference between the diets of the two was that more sweets and cereals were eaten by the group with the higher incidence of dental caries. Again investigating the diet, the author refers to some earlier work of his own. Children on Polynesian diets had almost no tooth decay while 98.2 per cent of the children on an oriental or high rice diet had tooth decay to a definite degree. Both of these diets were considered to be compatible with general good health. It was considered that the rice, having an acid residue, might lead to conditions in the mouth leading to dental caries to a greater extent than the Polynesian diet of taro, fruits, and vegetables which apparently yields an alkaline residue.

The possible roles of fluorides, oxalates, and vitamins A and K in the drinking water and diets of residents of Hawaii were commented on by the author. The drinking water of the territory of Hawaii is low in fluorides, containing only 0.02 and 0.2 parts per million of fluorine. Since sea foods are high in fluorides, Larsen postulated that some additional fluorides might be obtained from this source. No data were presented to indicate whether the amount of fluorides thus consumed might be appreciable or whether there was a variation between races in the consumption of sea foods.

This investigator discussed the role of oxalates in the diet as a possible caries-protecting agent with reference to the work of R. A. Gortner, C. M. McCay, J. S. Restarski, and C. A. Schlack (*J. Nutrition* **32,** *121 (1946)*) who demonstrated that the addition of oxalates to certain acid beverages appreciably reduced the degree of acid decalcification of rat molars. These workers stressed the fact that this type of acid decalcification did not necessarily have any relation to tooth decay. Later, other workers from the same laboratory demonstrated that the addition of oxalates to a cariogenic diet did not reduce the susceptibility of white rats or cotton rats to tooth decay (*Nutrition Reviews* **6,** *75 (1948);* S. R. Howell, C. A. Schlack, B. L. Taylor, and B. J. Berzinskas, *J. Dental Research* **27,** *136 (1948)*). However, Larsen has misquoted the former findings in such a way as to indicate a known similarity between this type of acid decalcification and tooth decay: "it has been

shown that the oxalates present in taro and absent in rice give a form of surface protection (in rats) that apparently provides as much decay resistance as fluorine."

The allusion to the possible importance of vitamins A and K in the prevention of tooth decay gives greater credence than is merited to experiments which are inadequately conducted and controlled. The only evidence quoted to support the role of vitamin A was that presented by N. P. Larsen, G. P. Pritchard, C. Wilbar, and A. L. Y. Ward (*Proc. Pacific Sci. Congress* **6,** *569 (1943)*) where each of 8 children was given 4 ounces daily of raw carrot juice for four months and each of 11 children was given 30 drops of oil containing 15,000 I. U. of beta-carotene per day for forty-three days. These children were examined at frequent intervals for nine months from the beginning of the experiment. No control children without any additions to their normal diet were examined during the same period. From the clinical examination, it was thought that there had been a reduced dental caries activity during the supplementation period and an increased activity during the following months. However, most dental investigators would feel that these experimental and observation periods were insufficiently long even for a well controlled experiment with large groups of children.

The speculation that vitamin K might be higher in the Polynesian diet was based on the presence of vitamin K in sugar cane. Here again the only data on the possible role of vitamin K as a caries-preventing agent in man consists of one experiment in which inconclusive results were obtained when vitamin K was incorporated into chewing gum (*Nutrition Reviews* **4,** *213 (1946)*). Animal experiments in which vitamin K was added to the diet did not indicate a protective effect.

The author concludes that despite conditions in which good general health was observed, severe tooth decay might be regularly seen and, conversely, severe tooth decay did not necessarily presuppose poor general health. The author has certainly made a massive survey of the incidence and possible factors relating to the incidence of dental caries in the Hawaiian Islands. However, it was impossible to do more than suggest what factors might be operative in causing and, conversely, in protecting children from caries. Although an attempt was made to separate the various population groups, this was not always entirely possible and even when it was, the results are difficult to interpret. The author pointed to the many variables which must be carefully studied to ascertain the role of race, dental hygiene, oral hygiene, and nutrition in the incidence of dental caries.

Probably the most serious fault of this presentation is the lack of data on the average ages and distribution of age and the distribution of boys and girls in the groups studied. It was stated that the 866 children from plantations were between the ages of 6 months and 18 years. No comment was made on the age range for the 104 new Filipinos nor for the 180 kindergarten children. One table in which the age incidence of decay is compared for the heterogeneous groups of Hawaiian and American children limits the data to permanent teeth only; in all other tabulations, no reference is made as to whether the number of DMF (decayed, missing, and filled) teeth refers to deciduous or permanent teeth only or to the mixed dentition. These data are all of the greatest value in any appraisal of the comparative dental caries experience of several groups and without which any presentation is largely meaningless. Undoubtedly the investigator had assembled these various data; it is regrettable that they were not presented so that the reader could evaluate the importance of the differences between groups which were discussed.

BIOCHEMICAL ASPECTS OF PTEROYLGLUTAMATES

In contrast to many other vitamins and trace substances, the biochemical role of folic acid is not yet clearly defined; folic acid stands as a necessary growth factor for certain bacteria and as a useful drug in the treatment of some macrocytic anemias, although not replacing the value of liver extract in the treatment of pernicious anemia. Nevertheless, several research reports indicate the directions taken to reveal the biochemical function of this substance.

Most prominent among possibilities for a biochemical function is that folic acid or a derivative is concerned with the biologic synthesis of the pyrimidine, thymine. It will be recalled that thymine, in large doses, was found to produce a definite but small hematologic remission in cases of pernicious anemia, sprue, and nutritional macrocytic anemia (*Nutrition Reviews* **4,** *333* (*1946*); W. B. Frommeyer and T. D. Spies, *Am. J. Med. Sci.* **213,** *135* (*1947*)). Other workers have shown that thymine and purines promote growth of Streptococcus faecalis R and Lactobacillus casei in the absence of folic acid in the medium. This and related work led J. L. Stokes (*J. Bact.* **48,** *201* (*1944*)) to the suggestion that folic acid functions as a coenzyme in an enzyme system engaged in the synthesis of thymine or related substance. There have been some objections to this hypothesis, but the further work of L. L. Rogers and W. Shive (*J. Biol. Chem.* **172,** *751* (*1948*)) adds weight to the theory of an intimate functional relation of folic acid and thymine. These workers studied the growth of L. casei with variations in the added purine, thymine, and folic acid, and in the presence of the folic acid inhibitor, methylfolic acid. Methylfolic acid showed a marked growth-inhibiting property which was not particularly affected by addition of thymine. However, when purines such as hypoxanthine were present, which themselves necessitated only a two- to threefold increase of the inhibitor to stop growth, then thymine addition produced a marked effect on the toxicity of the inhibitor. Under such conditions, ten to thirty times as much methylfolic acid was necessary to inhibit growth. An analysis of the results led these investigators to conclude that the probable function of folic acid was in an enzyme system engaged in the synthesis of purines or thymine. By supplying the products of the reactions catalyzed by folic acid, the inhibiting effect of methylfolic acid had presumably been by-passed.

Despite some objections to this type of "inhibition analysis," it is of interest that the general conclusion finds a parallel in a similar relation between the new vitamin B_{12} (*Nutrition Reviews* **6,** *245*, *291* (*1948*)) and thymidine, the nucleoside of thymine. A new factor from hog liver was particularly effective in overcoming the methylfolic acid growth inhibition of Leuconostoc mesenteroides 8293, and this factor had properties similar to that of thymidine. Furthermore, thymidine was found to replace vitamin B_{12} as a growth factor for Lactobacillus lactis Dorner, the test organism for vitamin B_{12}. These relations of folic acid, vitamin B_{12}, thymine, and thymidine are most interesting and have been reviewed (*Nutrition Reviews* **7,** *19* (*1949*)).

Perhaps in some way related to folic acid and purine metabolism is a xanthopterin oxidase described by H. M. Kalckar and H. Klenow (*J. Biol. Chem.* **172,** *349*, *351* (*1948*); **174,** *771* (*1948*)). This enzyme, as well as xanthine oxidase, is strongly inhibited by 6-pteridyl aldehyde. Another enzyme was noted which acted on an impure pteroylglutamic acid to produce a substance of similar physical properties, but which no longer possessed an inhibitory effect on xanthopterin oxidase.

Another, as yet apparently unrelated function of folic acid, lies in its peculiar relation with tyrosine (or other phenols) metabolism and ascorbic acid. It may be recalled that blood phenol levels are high in pernicious

anemia, and that liver extract treatment of this condition promptly reduces the excretion of phenolic substances (*Nutrition Reviews* **6,** *70* (*1948*)). Related is the report of G. Rodney, M. E. Swendseid, and A. L. Swanson (*J. Biol. Chem.* **168,** *395* (*1947*)) in which liver suspensions from folic acid deficient rats show an increased ability to oxidize tyrosine when pteroylglutamic acid is added. Since it is known that tyrosine metabolism is decreased in ascorbic acid deficiency, C. W. Woodruff and W. J. Darby (*Ibid.* **172,** *851* (*1948*)) were led to study the effect of pteroylglutamic acid on tyrosine metabolism in scorbutic guinea pigs. The diet fed was not only scorbutogenic but contained 5 per cent L-tyrosine. The twenty-four hour excretions of "tyrosyl" derivatives and keto acids were determined. Excretions of these substances reached high levels, but were promptly and markedly reduced upon administration of either ascorbic acid or pteroylglutamic acid. These results do not necessarily imply a relation between the two vitamins, ascorbic acid and pteroylglutamic acid, but implicate both in some phases of tyrosine metabolism.

B. C. Johnson and A. S. Dana (*Science* **108,** *210* (*1948*)) have reported an interesting interrelation between pteroylglutamic acid and ascorbic acid which might be related to the above findings. These investigators found that both growth and certain blood abnormalities in pteroylglutamic acid deficiency in rats were favorably affected by ascorbic acid administration. The deficiency was produced by feeding a synthetic diet plus a sulfa drug. Growth of the rats stopped in five weeks and a hemorrhagic condition around the mouth, nose, and paws was a frequent finding. Administration of 2 mg. of ascorbic acid daily reinstituted growth and the hemorrhagic condition disappeared. The average white cell count fell from 8000 to about 4000 in the deficient period, but rose to 7200 during two weeks of ascorbic acid therapy. This marked effect occurred in the granulocytes, lymphocytes, and monocytes. Ascorbic acid therapy also caused a significant formation of nucleated red blood cells, but the decline in hemoglobin and red blood cell count continued. After three weeks of ascorbic acid therapy, addition of pteroylglutamic acid to the diet brought about a marked reticulocyte response. The authors conclude that the leukopenia in the pteroylglutamic acid deficient rat can be effectively treated with ascorbic acid, and that ascorbic acid may function in the formation of normoblasts although pteroylglutamic acid is necessary for red blood cell maturation.

The biochemical role of folic acid is not nearly so well defined as that of several other vitamins, yet much progress has been made considering the relatively recent discovery of this factor. The research activity in this field, as indicated by the above reports, promises an early solution of this phase of the folic acid story.

UTILIZATION OF PROTEIN HYDROLYSATES

Despite a marked resurgence of interest in protein nutrition and particularly its use in the therapy of a number of diseases, a considerable amount of basic work has remained undone. In the last few years, however, many gaps have been filled by the work reported by J. B. Allison (*Am. J. Med.* **5,** *419* (*1948*)) from the Bureau of Biological Research at Rutgers University. In a statement of some of the basic concepts of protein metabolism in normal and protein depleted animals, Allison has drawn upon this experimental work at Rutgers.

In discussing nitrogen equilibrium and balance Allison points out that since there are no reserves of essential amino acids in the body, the amount of protein required to

maintain nitrogen equilibrium increases as the concentration of the essential amino acids in the protein decreases. This fact is particularly well illustrated by the failure to maintain nitrogen equilibrium when an acid hydrolysate of casein is used as a sole source of nitrogen for the dog. Destruction of tryptophan in acid hydrolysis leads to negative nitrogen balance which may be reversed, however, when an optimum amount of tryptophan is added to the hydrolysate. This method may be used for evaluating protein hydrolysates but is open to the objection, as the author points out, that the minimum amount of protein required to maintain nitrogen equilibrium decreases as protein depletion increases and is roughly inversely proportional to the amount of the protein depletion; thus, in a depleted dog, positive nitrogen balance may be obtained on a much lower daily protein intake than if the dog were previously well fed.

The relation between the protein intake and nitrogen balance has been expressed by Allison in a formula: $NB = K \times AN - NE_o$ where NE_o is the excretion of nitrogen on a protein-free diet, NB the nitrogen balance, and K a constant which has been called the nitrogen balance index of the protein fed. This formula has been previously discussed (*Nutrition Reviews* **4,** *29 (1946)*). Using these data for various protein sources, the nitrogen balance index for each protein may be readily calculated. Several nitrogen balance indices are presented, that for egg white and lactalbumin being 1.0. Casein is 0.8 while casein plus methionine is 1.2. Casein hydrolysate was found to be the same as casein. In the case of wheat gluten, known to be deficient in lysine, a low value of 0.44 was found whereas the same protein with a supplement of lysine gave a value similar to casein of 0.82. In determining comparable nitrogen balance indices for various proteins, normal, well fed dogs must of course be used, for were protein depleted dogs used, because of the better utilization of protein a higher nitrogen balance index would be observed. This is shown by the example given by the author of wheat gluten, with a nitrogen balance index of 0.45 in the normal animal, but 0.7 in the depleted animal. The enhancing effect of methionine upon the nitrogen balance index for casein is in agreement with the data of others and is true not only for the dog but also for the rat. In contrast, however, methionine has no nitrogen-sparing action in man, either in infants or adults (*Ibid.* **5,** *326 (1947)*).

In a similar manner with slight changes in the formula, nitrogen balance indices for protein hydrolysates given intravenously may be determined. Here again the nitrogen retention is greater in a protein depleted dog than in a normal dog. In each instance, for four protein hydrolysates the nitrogen balance index when the hydrolysate was given orally was much higher than when the hydrolysate was given intravenously.

The author has reviewed the evidence indicating the importance of maintaining an adequate caloric intake to insure maximum nitrogen retention. It is unnecessary to review this evidence here except to say that, as is well known, if the caloric intake falls below a certain critical level nitrogen retention decreases abruptly and negative nitrogen balance may ensue. The author likewise reviews the evidence for strepogenin or some other similar substance being necessary for the proper utilization of hydrolyzed protein given intravenously. Enzymatic hydrolysis, in experiments of the authors and of others, did not alter the strepogenin content of the resulting hydrolysate. However, even using synthetic amino acid mixtures, experiments indicated that peptides like strepogenin were not indispensable although at times might improve the utilization or retention of nitrogen.

Data indicating that there is an upper limit to nitrogen retention are presented. That is, increasing the nitrogen intake above a critical level does not further increase the nitrogen balance even in depleted dogs.

The positivity of the nitrogen balance which can be attained by feeding a complete protein such as whole egg is far greater than that which may be obtained when wheat gluten is fed.

With respect to the effect in the body of the depletion of protein by feeding a low protein diet or protein-free diet for a considerable period of time, Allison first discusses the effect on the plasma proteins. It is pointed out that there is a fall in the total protein, being largely due to a fall in albumin and gamma-globulin and with a slight increase in the alpha-globulin concentration. Associated with the body protein depletion was found an increase in the extracellular fluid volume together with a decrease in plasma volume. When protein is again fed to such a depleted animal "repletion" occurs and both body tissue proteins and plasma proteins are regenerated at an approximate ratio of 30 to 1.

In all, Allison has presented a clear and timely review of the work done by himself and his group at Rutgers and also by many others working in the field of protein metabolism.

N. F. Young (*Am. J. Med.* **5,** *586* (*1948*)) has also discussed protein hydrolysates, particularly with reference to chemical considerations in their selection for use in man. It is emphasized that in addition to the usual criteria for selecting parenteral products, protein hydrolysates must be derived from protein of high biologic value with all amino acids remaining in the product in the same pattern as in the protein and that the product must be nonantigenic, should contain a low concentration of dicarboxylic amino acid, be capable of a high rate of infusion, and be stable, that is, free from precipitation on standing. The low concentration of the dicarboxylic amino acids is of course indicated because of their nausea-provoking quality (*Nutrition Reviews* **3,** *312* (*1945*)). At present the best products available do not permit convenient administration of the large amounts surgical patients frequently require because the rapidity of administration necessary when large amounts are given regularly leads to nausea and frequently vomiting.

In the selection of an oral protein hydrolysate preparation, Young points out that in addition to biologic value, acceptability to the patient is of great importance. In addition, the preparation must not contain substances other than protein hydrolysates which might be harmful or at least not required, for example, sodium chloride which is present in large quantities in some of the protein hydrolysate preparations for oral use. Further studies on the oral use of protein hydrolysates have been recently reviewed (*Nutrition Reviews* **6,** *122* (*1948*)).

ASCORBIC ACID METABOLISM IN VITAMIN A DEFICIENT RATS

Since the demonstrations by B. Sure, R. M. Theis, and R. T. Harrelson (*J. Biol. Chem.* **129,** *245* (*1939*)) that depletion of vitamin A in the rat was followed by a fall in the concentration of ascorbic acid in the tissues, numerous studies of possible relations between these two vitamins have appeared. These investigators found an even more pronounced decrease in tissue ascorbic acid in deficiencies of riboflavin or thiamine. L. W. Mapson and S. E. Walker (*Brit. J. Nutrition* **2,** *1* (*1948*)) point out that most such studies have failed to control adequately the food intake of animals and, thereby, have failed to eliminate the effect of simple inanition in bringing about the reported changes in ascorbic acid in vitamin A deficient rats.

Mapson and Walker (*loc. cit.*) have studied the influence of vitamin A deprivation on tissue content of ascorbic acid, in both ad libitum and paired-feeding studies. These

investigations were further extended to the relation of vitamin A to the synthesis of ascorbic acid under the stimulus of chloretone. Blood ascorbic acid was measured as the dehydroascorbic acid by the method of J. H. Roe and C. A. Kuether (*J. Biol. Chem.* **147,** *399 (1943)*). The estimation of ascorbic acid in tissue extracts was made by a titration against 2,6-dichlorophenolindophenol. Tissue estimations of vitamin A were carried out by the antimony trichloride procedure after digestion with 5 per cent potassium hydroxide solution.

The investigations of animals on an unrestricted diet were carried out as follows: Male albino rats were fed a usual type of purified vitamin A deficient diet. One group received no supplement; the other was given 1500 I.U. of vitamin A weekly as halibut liver oil. Two to three weeks after growth ceased in the deficient animals, a group of them was killed for tissue analysis for ascorbic acid. Simultaneously, the rats which had received vitamin A were killed. The remaining group of vitamin A deficient rats was then supplemented with 1500 I. U. of vitamin A per rat per week. In accord with findings of other investigators, the ascorbic acid content of the livers of the vitamin A deficient rats was definitely reduced, both in concentration and total amount. Thus, the vitamin A deficient animals had 0.16 plus or minus 0.006 mg. of ascorbic acid per gram of liver tissue, while the vitamin A supplemented group had 0.24 plus or minus 0.012 mg. per gram of liver tissue. A similar difference was noted in the blood ascorbic acid and the expected pronounced differences were found in the vitamin A content of livers. Administration of vitamin A to the one group of deficient animals resulted in a definite increase in the concentration of ascorbic acid in both the liver and blood as well as definite increases in the vitamin A content of the liver.

Similar findings were obtained when male piebald animals were used instead of albino rats. However, the decrease in ascorbic acid in the blood of the vitamin A deficient male piebald was less than that observed in the vitamin A deficient male albino rat. The difference in blood levels of 0.29 mg. per cent between the vitamin A deficient and nondeficient piebald male rats was significant, however. On the other hand, piebald female rats exhibited no decreased concentration of ascorbic acid when subjected to vitamin A depletion. The finding of lower concentrations of ascorbic acid in the blood and liver of piebald female rats than in the corresponding tissues of male animals is in agreement with studies on the concentration of ascorbic acid in the blood of the two sexes of rats (E. N. Todhunter and T. J. McMillan, *J. Nutrition* **31,** *573 (1946)*).

These confirmed differences between the tissue concentration of ascorbic acid of the vitamin A deficient rat and the vitamin A-fed rat could be due to some impairment of ascorbic acid synthesis, to an excessive loss of ascorbic acid, or to such factors as inequality of food intake.

To test the possibility of excessive loss of ascorbic acid from the body, the investigators placed 60 to 70 g. piebald rats on a vitamin A deficient diet. One group received no additional vitamin A; the second received 1500 I.U. of vitamin A per rat per week. Twenty-four hour samples of urine were analyzed for ascorbic acid twice weekly. The study was continued for eighty days. No evidence was obtained of an increased excretion of ascorbic acid in the vitamin A deficient rat. There was, in fact, a gradual decrease in the daily excretion of ascorbic acid in the urine as the animals became more deficient in vitamin A. Thus, it was concluded that the observed differences in ascorbic acid contents of tissues of vitamin A deficient rats and their controls could not be explained by excessive loss of ascorbic acid through the kidney.

To test the effect of the inequality of food intake on ascorbic acid synthesis, 40 to 50

g. male albino rats were given the vitamin A deficient diet. One group received the diet alone; another group received the diet in amounts restricted to that of the animals in the first group, but, in addition, were fed vitamin A. Upon killing the animals, it was found that the ascorbic acid content of the liver in those animals receiving vitamin A with restricted food intake was identical with those deficient in vitamin A. However, a significant difference was found between the groups in the ascorbic acid content of the blood—the deficient animals having 0.38 plus or minus 0.025 mg. per 100 ml., the vitamin A-fed animals having 0.80 plus or minus 0.029 mg. per 100 ml. of ascorbic acid. A third group of vitamin A-fed, food-intake-restricted animals was permitted to eat ad libitum for one week. At the end of this time, the liver and blood ascorbic acid contents were significantly higher than the level in vitamin A-fed, food-restricted animals. Under the same conditions, piebald rats showed similar but less pronounced changes. Female rats, with their lower initial blood ascorbic acid contents, exhibited no changes attributable either to vitamin A intake or to total dietary intake.

The effect of chloretone on the synthesis of ascorbic acid in vitamin A deficient rats was also studied. These experiments were first made with male piebald animals fed ad libitum. It is to be noted that the workers had found that smaller differences occurred in the concentration of ascorbic acid of vitamin A deficient and paired vitamin A-fed piebald rats than between albino animals under similar conditions. When chloretone was administered to male piebald rats fed ad libitum on the basal diet, with and without supplements of vitamin A, the amount of ascorbic acid excreted rose sharply in both groups but was less in the depleted animals. Thus, the peak excretion for the ad libitum vitamin A-fed rats was 40 mg. per day compared with 15.5 mg. per day for the deficient rats. Similar, but lower, responses to chloretone were observed in female piebald rats.

Similar experiments were made on pair-fed male piebald rats, with and without vitamin A supplements. In these pair-fed animals, the mean rise in ascorbic acid excretion at the peak following chloretone was 6.9 mg. per day for the deficient group and 6.3 mg. per day for the vitamin A-fed group. The administration of chloretone increased the liver and blood concentration of ascorbic acid in rats irrespective of their vitamin A status.

These studies would have been easier to interpret had the added variable of differences between piebald and albino rats been avoided. Further, it would appear that in some of the groups the numbers of animals studied were fewer than one would desire for drawing decisive conclusions. Despite these limitations, the studies have demonstrated that variations in food intake alone can account for some of the differences in tissue ascorbic acid content which have been ascribed in the past to vitamin A deprivation. It was also demonstrated that at least in piebald rats there was no excessive loss of ascorbic acid through renal excretion when the animals were subjected to vitamin A depletion. It was further established that impairment of ascorbic acid synthesis by vitamin A deficient rats under the stimulus of chloretone was due in part to restriction in total food intake and not to vitamin A intake specifically. However, these studies do not appear to preclude an effect of vitamin A on ascorbic acid synthesis.

Of unusual interest in this investigation is the observation that the concentration of ascorbic acid in the suprarenal glands remained constant throughout despite deprivation of vitamin A, restriction of dietary intake, or administration of chloretone.

J. Mayer and W. A. Krehl (*Arch. Biochem.* **16,** *313 (1948)*; *Nutrition Reviews* **6,** *224 (1948)*) have also reported scorbutic symptoms in vitamin A deficient rats.

NUTRITION IN ADOLESCENCE

The nutritional requirements of growth and development during childhood and adolescence have been reviewed (*Nutrition Reviews* **5,** *124 (1947)*). Most studies which have been made have attempted to define the normal rate of growth and development but have stressed the wide variations in the normal. J. A. Johnston (*J. Am. Med. Assn.* **137,** *1587 (1948)*) in discussing the nutritional problems of adolescence likewise emphasizes the wide variations in the normal during this period of life. In a group of 13-year-old girls, all presumably normal, standing heights gained in a year varied from 1 to 4 inches and the gain in weight varied from 6 to 30 pounds. The wide variation in the onset of the menses was likewise illustrative of the difficulty in defining the normal at this age. The author points out, however, that when the same physiologic age group is used the individuals may be compared fairly accurately and predictions become possible. Thus the correlation of growth with sexual maturity rather than chronologic age becomes an important concept in assessing the nutritional status of the adolescent.

The author points out the marked increase in growth rate just preceding sexual maturity, both in girls and boys, and a marked reduction in growth immediately thereafter. Along with this goes an increased requirement for nutrients and an increased retention before puberty followed by a decreased retention and presumably requirement. This is true particularly of the calcium requirement and retention and also of the requirement for vitamin D. In respect to vitamin D, it was estimated that during this period of rapid growth the need is probably twice as great as at any other time in life except perhaps during the middle of the first year. In one of his previous studies the author found that instinct was a reliable guide to the amount of food required and to its character. He emphasizes, however, that the protein and calcium needs will be satisfied by the inclusion in the daily diet of a quart of milk, an egg, a serving of cereal, three slices of bread, two large servings of meat, fish, or cheese, and an egg or milk dessert. Such a diet it is estimated would provide, if nothing else were supplied, approximately 100 g. of protein and approximately 1.7 g. of calcium.

The problem of obesity in adolescence is considered and emphasis is placed on the fact that although it is necessary to limit the caloric intake in obese adolescents as well as in obese adults, it is particularly necessary in the child to estimate the emotional and social difficulties as they are frequently the basis of the overeating and, hence, obesity. An apparently very adequate 1400 calory, 20 per cent protein diet is included in the article. Such a diet furnishes large amounts of protein, calcium, and vitamin D. The author emphasizes that rarely is obesity in adolescence due to thyroid deficiency. This is well indicated by the advanced intellectual age and bone age in these individuals although their social and emotional maturity has usually been retarded.

Another problem in adolescence, particularly of the obese child, is that of slipping of the upper femoral epiphysis. The author believes that this condition may be due in part to the markedly increased requirements for vitamin D during the period of rapid growth during adolescence.

The problem of tuberculosis in the adolescent is given consideration by the author. In studies of his own, with others, of 1100 Detroit children it was found that deficits of body nitrogen apparently existed in these children even though they were almost all found in the minimal stage of tuberculosis. It was also felt that the decreased retention of nitrogen and calcium at the menarche in girls was a contributing factor to the re-

activation of the tuberculous lesion. This the author gives as additional support for the increased needs of protein, calcium, and vitamin D during the period of adolescence.

In a discussion of the menstrual irregularities in adolescence, the author found four separate categories with respect to etiology, i.e., nutritional, emotional, hormonal, and organic. With respect to nutritional factors, both obesity and malnourishment, presumably undernourishment, are found to be important contributors to irregularities of the menstrual cycle. In both instances, regulation of the diet to the needs of the child will usually restore the menses to regularity. Again, thyroid is not necessarily indicated in the obese group. As evidenced by slow pulse, small pulse pressure, and diminished metabolic rate with fatigue and pallor, approximately one quarter of 50 high school students were judged to require thyroid. Very small quantities of thyroid should be given daily, however; in fact rarely was it necessary to use more than 0.5 grain daily. When too much thyroid is administered, the catabolic nature of the hormone comes into play. Finally, the author stresses that if the menstrual irregularities persist despite what may be considered adequate therapy, gynecologic examination may be necessary. In the series of high school girls studied by Johnston, 6 of them had organic lesions presumably associated with or perhaps causing the menstrual irregularities.

VITAMIN THERAPY OF GASTRITIS IN MAN

The possibility that stomach lesions in man may arise on the basis of chronic nutritional deficiency has often been proposed but few data are at hand to substantiate it. Stomach lesions, chiefly ulcerous in variety, have been produced in experimental animals who have been fed a deficient diet for long periods of time. Deficiencies of various nutrients have been implicated, such as choline (*Nutrition Reviews* **1,** *396 (1943)*), calcium (*Ibid.* **2,** *13 (1944)*), and various vitamins of the B-complex (*Ibid.* **2,** *67 (1944)*). Little work has been done in man, although in pernicious anemia C. M. Jones, E. B. Benedict, and A. O. Hampton (*Am. J. Med. Sci.* **190,** *596 (1935)*) demonstrated prompt improvement and disappearance of atrophic gastritis following liver therapy. Others have indicated that atrophic gastritis may be at least in part a nutritional deficiency but fails to respond to administration of the vitamin B-complex and vitamin A.

L. H. Berry and T. J. Cole (*J. Am. Med. Assn.* **138,** *485 (1948)*) have reported a long-time gastroscopic study of 40 patients with atrophic gastritis; 16 were held as controls for periods varying from one to eighteen months. Thirty-six were treated in groups with injections of the vitamin B-complex, injections of liver extract, injections of liver extract plus iron, injections of liver extract and hydrochloric acid by mouth, injections of niacin or, finally, administration of dilute hydrochloric acid by mouth alone. The patients were all of a low economic status and it is stated that their food intake was considerably below normal standards, although data on dietary intake are not presented. Plasma ascorbic acid levels were below 1 mg. per 100 ml. in 95 per cent of the cases and many had low-grade hypochromic anemia and varying degrees of loss of weight. Physical signs suggesting specific deficiency states were apparently uncommon, although the authors give no figures as to their incidence. Particularly disappointing is the lack of specific mention of tongue changes or pharyngeal abnormalities.

The extent of gastric mucosal atrophy was graded from 4 plus to 1 plus and absence of atrophy listed as zero. One or more control gastroscopies were performed on each in-

dividual before therapy was begun. All patients in the treated groups had either 3 plus or 4 plus atrophy before therapy was begun. In the first group of 10 patients given intramuscular injections of vitamin B-complex, atrophy disappeared in 7 of the 10 cases studied, in 4 of whom it returned to some degree; the other 3 cases were improved. Twelve of the 18 cases given intramuscular liver extract injections likewise had complete disappearance of atrophy. Although it is stated by the authors that the addition of iron or dilute hydrochloric acid to the liver extract therapy did not improve the results, it would appear that the number of cases in each of these two groups was too small to be significant. Another small group of patients, 4 in number, was given daily intramuscular injections of 50 mg. of niacin. Two showed complete disappearance of atrophy; one improved markedly, and one improved either slightly or not at all. Again this group would appear to be too small for definite conclusions to be drawn. The liver extract used "contained 5 pernicious anemia units per cc." but was otherwise undefined. The composition of the vitamin B-complex given to the first group of 10 patients was likewise not given by the authors.

In addition to the objective evidence of improvement observed by repeated gastroscopy there was subjective improvement in symptoms in most of the patients which was dramatic after administration of liver extract, the vitamin B-complex, and niacin, but less so after the dilute hydrochloric acid regimen. It is unfortunate that the control periods before therapy was begun were not longer, nevertheless the objective improvement observed in the gastric mucous membranes was striking. It is to be hoped that a similar group of patients with atrophic gastritis will be observed and carefully examined for evidence of nutritional deficiency by the clinical and laboratory methods available. More complete dietary history would likewise seem to be indicated.

ROLE OF PYRIDOXINE IN TRANSAMINATION

Transamination, the biologic process of transferring amino groups from appropriate amino acids to keto acids forming other amino acids, is of obvious importance in protein metabolism, and the recognized role of the vitamin pyridoxine in the process (*Nutrition Reviews* **3,** *291* (*1945*); **4,** *232* (*1946*)) evokes further interest from the nutritionist. Pyridoxal and pyridoxamine, in conjunction with the biologic phosphorylating agent adenosinetriphosphate, have both been recognized as active in the coenzyme functions of pyridoxine. Pyridoxal phosphate has been most commonly used as the coenzyme for *in vitro* studies of tyrosine decarboxylase and transaminase.

In contrast to the known mechanisms by which some of the known coenzymes function, the type of reaction which pyridoxal phosphate might undergo in its function has not been clear. However, E. E. Snell (*J. Am. Chem. Soc.* **67,** *194* (*1945*)) had demonstrated the interconversion of pyridoxal and pyridoxamine by heating with the appropriate amino or keto acid. He therefore suggested that the transfer of amino groups which occurs in transamination involves the same type of interchange within the coenzyme molecule, represented by the reaction pyridoxal phosphate $\rightleftharpoons$ pyridoxamine phosphate. This suggestion was in conformity with the accepted "carrier" type role for coenzymes and was therefore an attractive hypothesis. Apparent support of the hypothesis was provided by the work of W. W. Umbreit, D. J. O'Kane, and I. C. Gunsalus (*J. Bact.* **51,** *576* (*1946*)) in which pyridoxamine phosphate was found to be active as a coenzyme of transaminase, and by that of S. R. Ames, P. S. Sarma, and C. A. Elveh-

jem (*J. Biol. Chem.* **167,** *135 (1947)*) in which pyridoxamine phosphate served to stimulate the depressed transaminase activity of liver homogenates prepared from pyridoxine deficient animals.

According to Snell's hypothesis of the coenzyme function, pyridoxal and pyridoxamine phosphates should be equally active in transaminase systems since they are interconvertible during coenzyme function. However, in the course of studies by W. W. Umbreit, D. J. O'Kane, and I. C. Gunsalus (*J. Biol. Chem.* **176,** *629 (1948)*) it was noted that one of their transaminase preparations from Streptococcus faecalis R and one from pig heart could not be activated by pyridoxamine phosphate, although pyridoxal phosphate was active. Thus some doubt was cast on the theory of amino group transfer within the coenzyme molecule.

These investigators recognized that experiments in which pyridoxamine was found active dealt with biologic material so complex that the amine might have been converted to pyridoxal phosphate, and that only the latter might be the active coenzyme. Thus the activity of pyridoxamine was put to a more critical test by using a more purified transaminase preparation, one freed of coenzyme and other enzymes which might effect a conversion of pyridoxamine to pyridoxal. For this purpose the glutamic-aspartic transaminase of pig heart was purified, and the formation of oxalacetate from aspartic acid and alpha-ketoglutarate used as a test of its activity. Pyridoxal phosphate proved to act as a coenzyme and catalyzed the reaction, but pyridoxamine phosphate was inactive.

The pyridoxamine phosphate used in these experiments was prepared by heating pyridoxal phosphate with glutamic acid, and the possibility remained that the product is not the active pyridoxamine compound postulated as the coenzyme. It was noted however that the compound so produced had growth-promoting properties identical to those of the pyridoxamine phosphate prepared by direct phosphorylation of pyridoxamine.

Thus the conclusion was reached that pyridoxamine phosphate is not a coenzyme in the transaminase system, and the otherwise attractive theory of amino group transfer within the coenzyme molecule is not substantiated. Unfortunately, no other plausible theory for the action of pyridoxal phosphate as a coenzyme of tyrosine decarboxylase, tryptophanase, or transaminase has been offered.

OXIDATION OF GLUCOSE BY NORMAL AND ALLOXAN-DIABETIC RATS

The nature of the metabolic defect in diabetes has been the subject of prolonged debate and investigation since the recognition of the disease itself and the specific therapy that would reduce the severity of the disease. Rigid proof that there is an impaired capacity of the diabetic organism to utilize glucose by converting it to carbon dioxide through normal physiologic pathways still waits. The increased availability of compounds, including glucose, labeled with radioactive carbon in known positions makes it possible to study with greater accuracy the fate of carbon-containing compounds in the animal body. A diabetic syndrome can be produced in the laboratory rat by the injection of alloxan and is characterized by extensive glucose excretion in the urine, as well as by excessive urination, and voracious eating accompanied by considerable decreases in body weight.

The utilization of radioactive glucose by alloxan-diabetic rats has been compared with that of normal rats by D. B. Zilversmit, I. L. Chaikoff, D. D. Feller, and E. J. Masoro (*J. Biol. Chem.* **176,** *389 (1948)*). Diabetic rats were produced by the single injection of 200 mg. of alloxan per kilogram of body

weight intraperitoneally or by the injection of 50 mg. of alloxan per kilogram of body weight per day intravenously. Glucose was determined in urine samples by oxidation with potassium ferricyanide and subsequent titration with ceric sulfate. Blood glucose was determined by the same method on an aliquot of protein-free filtrate. Glucose labeled with radioactive carbon in the third and fourth positions was prepared from tobacco leaves which had been illuminated in the presence of carbon dioxide tagged with radioactive carbon ($C^{14}O_2$). The labeled glucose was obtained by the acid hydrolysis of starch, followed by fractional crystallization of the soluble sugars. During the period of observation after the intraperitoneal injection of radioactive glucose, the rats were kept in individual all-glass cages ventilated with carbon dioxide-free air. Exhaust air from the cage was passed through a column of carbonate-free sodium hydroxide solution to absorb all expired carbon dioxide. At the end of the experimental period, the carbon dioxide content and the radioactivity of this solution were determined. The amount of total glucose and the amount of radioactive glucose in the urine excreted during the same periods were determined. The amount of radioactive glucose was measured by two methods: (1) determination of the amount of carbon dioxide radioactivity evolved in the fermentation of the glucose in an aliquot of urine by a yeast (Torula monosa), and (2) the formation of glucosazones from an aliquot of urine, oxidation of the isolated glucosazones to carbon dioxide followed by an evaluation of the radioactivity of this sample of carbon dioxide. The latter method was much less sensitive because of the threefold dilution of the carbon of glucose by the carbon from phenylhydrazine. The values obtained by these two methods were in fairly good agreement. This is of real importance since it implies that the specific activity during metabolism of the third and fourth carbon atoms in the glucose chain is not different from that of the other four carbons and excludes the possibility that the expired carbon dioxide is derived only from one or two specific carbon atoms in the glucose molecule.

When normal adult rats were injected intraperitoneally with either 100 mg. of labeled glucose or with 1 g. of labeled glucose per kilogram of body weight, between 40 and 60 per cent of the administered radioactive carbon was expired as carbon dioxide within six hours. In one rat which expired 55 per cent of the injected radioactive carbon in the first six hours after injection, only an additional 11 per cent was recovered in the carbon dioxide expired during the next eighteen hours. This higher early conversion of labeled glucose to carbon dioxide indicates that the labeled glucose within the rat is diluted during the course of this period by unlabeled glucose from the diet or from endogenous sources.

Diabetes had existed in the rats used for metabolic studies for ten to seventy days prior to the injection of labeled glucose, during which time there had been considerable weight loss, sugar excretion of 5.6 to 11.4 g. per day, and excretion of 50 to 120 ml. of urine per day. The 5 diabetic rats used in this study oxidized only 11 to 24 per cent of the injected labeled glucose in the first six hours thereafter. During this period, these rats had simultaneously excreted 30 to 45 per cent of the labeled glucose. Thus, these rats had metabolized a rather considerable amount of the labeled glucose which was available before urinary excretion. Little labeled glucose was excreted after twelve hours.

Since the diabetic rats excreted such high percentages of labeled glucose in the first six hours of the experimental period, no fair basis existed for comparison of the metabolic capacities of normal and alloxan-diabetic rats to oxidize the administered labeled glucose. Bilateral nephrectomy was used as a possible procedure for making the full amount of injected glucose available to metabolic processes. Fifteen minutes after

completion of the surgical procedures, each rat was injected intravenously with an amount of tagged glucose equivalent to 1 g. per kilogram of body weight. A dose of this size was sufficient to produce comparable initial values for the specific activities of circulating glucose in both normal and diabetic rats, thus minimizing the differences in plasma glucose between normal and diabetic rats in the initial period of the experiment. The amount of labeled carbon dioxide exhaled during the entire six hour experimental period did not differ significantly between nephrectomized normal and nephrectomized alloxan-diabetic rats. However, in the early period, from a half to four hours, 2 of the 5 diabetic rats exhaled appreciably less labeled carbon dioxide than any of the 4 normal rats. On the contrary, during the period from four to six hours, all 3 diabetic rats exhaled considerably more radioactive carbon than either of the control, nephrectomized rats. Plasma glucose levels in the latter group had returned to approximately normal values of 125 to 157 mg. per cent, while the plasma glucose of the nephrectomized diabetic rats at necropsy ranged between 700 and 1060 mg. per cent.

From the data in nephrectomized rats, it would seem that the alloxan-diabetic rat had about the same ability to convert glucose to carbon dioxide as the normal rat as judged by the total amount of radioactive carbon dioxide expired in the first six hours. However, the low rate of conversion during the early hours by diabetic rats and the greatly increased conversion toward the end of the period gives the suggestion that the conversion process in the diabetic rat does not occur through the same basic processes or else is dependent upon a higher plasma glucose level for maximum operation of the process. The accumulation of plasma glucose in the diabetic rats which were nephrectomized would seem to indicate an appreciable inability of these animals to utilize glucose for one or more functions other than the conversion to carbon dioxide. Investigation of other pathways for the utilization of glucose with parallel procedures gives promise of profitable and enlightening results.

As judged by these investigations, the ability of the alloxan-diabetic rat to convert glucose to carbon dioxide did not differ enough from that of the normal rat to be measured by available procedures. Even in the intact rat where large amounts of glucose were being excreted throughout the period when measurements of the radioactivity of the expired carbon dioxide were being made, rather large amounts of the labeled glucose were being converted to carbon dioxide. The rapidity of glucose excretion by the diabetic rats seemed to be the largest factor in preventing the amount of glucose converted to carbon dioxide from approximating that achieved by the normal rats.

OCULAR CHANGES IN DEFICIENCIES OF RIBOFLAVIN OR TRYPTOPHAN

The appearance of cataract and other ocular changes, including corneal vascularization, was described in vitamin G deficiency by P. L. Day, W. C. Langston, and C. S. O'Brien (*Am. J. Ophth.* **14,** *1005 (1931)*). Evidence for the identity of riboflavin as the vitamin G factor responsible for these ophthalmologic changes was provided by P. L. Day, W. J. Darby, and W. C. Langston (*J. Nutrition* **13,** *389 (1937)*). Similar ocular changes result from tryptophan deficiency (J. R. Totter and P. L. Day, *Ibid.* **24,** *159 (1942)*) as well as deficiencies in some of the other essential amino acids (*Nutrition Reviews* **6,** *244 (1948)*). Despite numerous studies of these conditions, a satisfactory explanation of the mechanism of development remains to be found.

A. Pirie (*Brit. J. Nutrition* **2,** *14 (1948)*) has reported additional observations on the

eye in riboflavin and in tryptophan deficiency. She notes that despite definite retardation of growth of the whole animal in both deficiencies, measurements of the eyeball indicated that this organ continued at a normal rate of growth. The result was that the deficient animals had relatively large eyes in undersized bodies. That this phenomenon is not grossly apparent is due to recession of the eyeball in the deficient animal. This recession is permitted by the underdevelopment of the Harderian gland. These glands were approximately half normal size in deficient rats. Sections of the glands from both riboflavin and tryptophan deficient animals showed dense lymphocytic infiltration replacing portions of the glandular tissue.

This underdevelopment of the Harderian glands suggested that the corneal vascularization seen in riboflavin and tryptophan deficiency might be due to the lack of Harderian secretion. Accordingly, the glands were removed from the right sockets of 4 healthy rats. The eyes were observed with a biomicroscope for a period of two months subsequently. The cornea remained clear and bright with no signs of drying or vascularization. It may be concluded that loss of Harderian secretion is not a major factor in the production of corneal vascularization in these deficiencies.

Estimations of the ascorbic acid content of lenses and Harderian glands from the tryptophan deficient animals were made by the 2,6-dichlorophenolindophenol titration. The only observed change from normal in ascorbic acid content was a definite lowering of ascorbic acid in the Harderian glands of the tryptophan deficient rats. The magnitude of this lowering may be appreciated from the mean value of 46 mg. per 100 g. of Harderian gland from animals on the tryptophan deficient diet as contrasted to a mean value of 80 mg. per 100 g. of Harderian gland for the animals on the same diet supplemented with 0.2 per cent dl-tryptophan. Inasmuch as these animals were fed ad libitum, it is impossible to judge whether this decrease in tissue content of ascorbic acid in tryptophan deficiency is a specific effect or one due to partial inanition, such as has been demonstrated for the ascorbic acid changes observed in vitamin A deficient animals (*Nutrition Reviews* **7**, *52* (*1949*)).

ACTIVITY OF PTEROYLHEPTAGLUTAMATE IN SULFA-TREATED RATS

Some time before clarification of the identity of the several pteroylglutamates, J. R. Totter and co-workers (*J. Biol. Chem.* **152**, *147* (*1944*)) observed that the distribution of the factor (folic acid) which stimulates growth of Streptococcus lactis R did not parallel the vitamin M activity of a series of foodstuffs. The vitamin M activity was measured by the protection which the supplement provided against nutritional cytopenia in the monkey. Certain of these substances contained but little S. lactis R activity, but were quite active in tests on monkeys.

Later work from the same laboratories (M. E. Mallory, V. Mims, J. R. Totter, and P. L. Day, *J. Biol. Chem.* **156**, *317* (*1944*)) demonstrated a similar lack of relation between the content of preformed S. lactis R-stimulating substance in a series of materials and the growth-promoting and leucocytopoietic effects of the same supplements when fed to rats on a diet containing succinylsulfathiazole. They also reported an increase in S. lactis R activity upon treatment of some of these foodstuffs with an enzyme preparation from fresh rat liver. The total S. lactis R activity so measured more nearly paralleled the leucopoietic effect of the materials.

These findings led the Arkansas investigators to conclude that the same factor protected the sulfa-treated rat and the vitamin M deficient monkey. It was sug-

gested that the nutrients concerned consisted of substances which either stimulated S. lactis R or which could be enzymatically converted into S. lactis R-stimulating substances. The latter compounds were termed "potential S. lactis R factor."

Following the revelation of the structures of the pteroylglutamates, the correctness of the above interpretations seemed evident. Experimental demonstration of the activity of the crystalline pteroylheptaglutamate (a potential S. lactis R factor) in the sulfa-treated rat has been provided by M. E. Swendseid, R. A. Brown, O. D. Bird, and R. A. Heinrich (*Arch. Biochem.* **16,** *367 (1948)*).

These latter workers used weanling rats on a diet containing 18 per cent purified casein and 2 per cent sulfasuxidine. The remainder of the composition of the diet followed standard lines for experimental rations of this sort. No pteroylglutamic acid (PGA) was included. Leucocyte and granulocyte counts were made weekly. When the total granulocyte count fell to 250 cells or less per cubic millimeter, test supplements were administered. The activity of a material was measured on a basis of the amount required to bring about an increase in cell counts four days after therapy to levels of 7500 total leucocytes and 2000 granulocytes per cubic millimeter. In addition to hematologic studies, the workers also determined the urinary excretion of PGA preceding and following the administration of the supplements. The supplements given were graded amounts of PGA, crystalline pteroylheptaglutamate, and concentrates of the heptaglutamate containing conjugase inhibitor. Comparisons were made of the effectiveness of the two glutamates by both oral and parenteral routes.

Thirty micrograms of PGA, orally or parenterally, induced a standard response. Twenty micrograms of PGA orally failed to induce a standard response. Orally administered crystalline pteroylheptaglutamate in doses equivalent to these two levels of PGA proved of equal activity with the monoglutamate. When given parenterally, however, 30 micrograms equivalent of pteroylheptaglutamate failed to produce standard responses in the leucopenic rat. Parenteral doses of the crystalline heptaglutamate corresponding to 40 micrograms of PGA resulted in a standard response.

Simultaneous administration of conjugase inhibitor and heptaglutamate orally to rats did not lessen the hematologic response to the pteroylheptaglutamate. This latter observation is somewhat surprising since the conjugase inhibitor did have an effect of reducing the excretion of PGA by the rats following oral administration of the conjugate. The average twenty-four hour urinary excretion on the day of supplementation was 3.20 micrograms for rats given crystalline conjugate without inhibitor and 1.60 micrograms for rats fed the same quantity of conjugate plus inhibitor. Thus, though the inhibitor decreased the urinary excretion of PGA, it did not influence the utilization of the heptaglutamate for leucocyte maturation and production. This observation makes one question the validity of the prevalent view that urinary excretion necessarily reflects the physiologic availability of a vitamin. Also, it suggests that the hematopoietic activity of pteroylglutamates might not be dependent upon their being broken down into PGA. On the other hand, it is consistent with the interpretation that the heptaglutamate is modified in the gastrointestinal tract.

The importance of this study is threefold: It emphasizes the complexity of the interpretation of data such as that previously reviewed (*Nutrition Reviews* **6,** *125 (1948)*) on urinary excretion of these vitamins; it verifies conclusions from work on crude preparations regarding "potential S. lactis R factor" in foodstuffs; and it reaffirms the necessity of measuring total pteroylglutamates of foodstuffs in analyses designed for preparation of food tables.

NOTES

William John Dann

The death of W. J. Dann of Duke University School of Medicine on December 5, 1948, brought to a premature end one of the more forceful influences in nutrition. Dann was the true experimentalist with "a straight, clear, and precise mind." He reveled in frank, honest discussion; he was ever impatient with ambiguous or illogically designed studies or with conclusions based upon emotional attitudes rather than scientific data. He possessed an acute awareness of the social responsibilities of science in general, of nutrition in particular. These traits, coupled with his generous cooperation with other workers, profoundly influenced his associates. This influence was readily apparent to one within the environment of Duke University; it was palpably obvious to those of us who had the privilege of his association as a member of the staff of *Nutrition Reviews*; it was exerted anonymously upon others through his many contributions to this journal and through his editorial comments on papers submitted to the *Journal of Nutrition*, on whose editorial board he served.

Dann was trained at Cambridge from which university he held both the Ph.D. and Sc.D. degrees. He moved to Duke University in 1934 as a Beit Memorial Fellow. He became successively Assistant Professor, Associate Professor, and Professor of Nutrition in the Department of Physiology of Duke University School of Medicine. He was a member of the American Institute of Nutrition and the American Society of Biological Chemists.

Dann's earlier contributions dealt largely with the physiology of vitamin A, especially as related to the maternal-fetal transfer. In collaboration with K. A. Evelyn, he devised the widely employed Dann-Evelyn method for determining vitamin A. In more recent years his investigations dealt primarily with niacin. With P. Handler he defined a chemical method for the determination of niacin and contributed to the knowledge of the distribution of niacin in foods. His contributions to knowledge of niacin metabolism were many, among which may be mentioned the demonstration of the synthesis of niacin by the chick, the metabolic relations of niacin in the dog, and the identification of dinicotinylornithine as a metabolite in the chick. Finally, shortly over a year ago there appeared under his editorship the very valuable symposium "Estimation of the Vitamins."

The science of nutrition is poorer because of the loss of W. J. Dann. The staff of *Nutrition Reviews* is saddened by the death of one of its family.

Vitamin Fortification Of Milk

The Food and Nutrition Board of the National Research Council adopted a statement this past year intended to clarify the Board's position with regard to fortification of milk. The statement has been published by the American Medical Association, Council on Food and Nutrition, together with an affirming note from the latter organization (*J. Am. Med. Assn.* **138,** *749 (1948)*). Quotations from the National Research Council statement follow. "The Board favors fortification of milk with vitamin D to the level of 400 U.S.P. units to the quart when the milk is used as a source of this vitamin . . . fortification of milk with vitamin A is deemed unnecessary as a general public health measure." The Board does not "now intend that its decision . . . should be interpreted as grounds for prohibiting investigation and market trials with milk modified by the addition of appropriate ingredients in reasonable amounts."

Folic Acid and Neurologic Changes in Pernicious Anemia

Considerable attention has been given to the status of folic acid in the treatment of

pernicious anemia (*Nutrition Reviews* **5,** *208, 274 (1947);* **6,** *90, 114, 245, 291 (1948)*). It has been observed repeatedly that not only may folic acid fail to halt progression of the neurologic changes associated with the disease, but actually may increase them.

Further evidence for the failure of folic acid to halt neurologic disturbances is given by S. D. Jacobson, L. Berman, A. R. Axelrod, and E. C. Vonder Heide (*J. Am. Med. Assn.* **137,** *825 (1948)*). Two patients with pernicious anemia in relapse were treated with varying but rather large amounts of folic acid (10 to 65 mg. daily). Both had evidence of combined systems disease before therapy. A maximum reticulocyte response occurred at the expected time following folic acid therapy and the hemoglobin and red blood cell counts began to rise. Approximately sixty and one hundred and twenty days after folic acid therapy was begun and while 10 mg. of the material were being administered daily orally, neurologic symptoms and signs reappeared, progressed rapidly, and became severe. Both patients were then given liver extract instead of folic acid, but in only one is its effect upon the neurologic lesions given, in this case remission.

The authors suggest that further clinical trials be made with larger amounts of folic acid to observe whether a progression of the neurologic changes can in this way be prevented. The evidence indicates that this would not be a safe or effective procedure. Since liver extract is at least known to halt progression of neurologic disease and often to effect a complete remission, this material would still seem to be the indicated material for the treatment of pernicious anemia. The discovery of vitamin B_{12} (*Nutrition Reviews* **6,** *245, 291 (1948)*) and its dramatic clinical effect in the treatment of pernicious anemia, both for the blood and neurologic disturbances, may in time supplant the use of liver as the therapeutic agent of choice in pernicious anemia.

Recent Nutrition Books

Experimental Immunochemistry. Elvin Kabat and Manifred Mayer. Charles C. Thomas, 301–327 East Lawrence Avenue, Springfield, Illinois. 1948. Pp. 567. Price: $8.75.

Feeds and Feeding. Ed. 21. Frank B. Morrison. The Morrison Publishing Co., Ithaca, N. Y. 1948. Pp. 1207.

Man and Animals. What they Eat and Why. (*A Manual of Nutrition*). Ben Dawes. Longmans, Green and Co., Inc., 55 Fifth Avenue, New York, N. Y. 1948. Pp. 100. Price: $2.00.

Men and Hunger—A Psychological Manual for Relief Workers. Harold S. Guetzkow and Paul H. Bowman. Brethren Publishing House, Elgin, Ill. 1946. Pp. 72.

Public Health in the World Today. Edited by James Stevens Simmons. Harvard University Press, Cambridge 38, Mass. Publication: April 5, 1949. Price: $5.00.

Relative Cost of Nutrients in a Serving of Food. Mabel A. Rollins. Cornell University Agricultural Experiment Station Bulletin 845. October 1948. Pp. 65.

Selected Works of Henry C. Sherman. Compiled by the Sherman Testimonial Volume Committee, Columbia University. The MacMillan Co., 60 Fifth Avenue, New York, N. Y. 1948. Pp. 1056. Price: $5.00.

The Frozen Food Cook Book. Jean I. Simpson and Demetria M. Taylor (with the technical assistance and cooperation of The Frozen Food Foundation.) Simon and Schuster, Inc., Rockefeller Center, 1230 Sixth Avenue, New York 20, N. Y. Copyright, 1948, by the Frozen Food Foundation, Inc. Pp. 493. Price: $2.95.

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